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Computational analyses of JAK1 kinase domain: Subtle changes in the catalytic cleft influence inhibitor specificity

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ABSTRACT

The Janus kinases (JAKs) are a family of intracellular non-receptor tyrosine kinases which transmit signals by phosphorylation of downstream substrates. A myriad of cytokines can trigger the JAK-STAT pathway which influences immune response, embryonic development, and cellular transformation. Here, we built a comparative model for Jak1 based on the crystal structure of Jak2 (PDB code:2B7A) and Jak3 (PDB code: 1YVJ) using the InsightII package. 3D-Profile and stereochemical analysis further verified the validity of the proposed structure. Adenosine 5′-triphosphate (ATP) was then docked into its catalytic cleft. Although the shape of Jak1 kinase cleft is fairly similar to that of Jak3, we observed minute changes in the key residues of the binding interface which influenced the docking of a specific Jak3 inhibitor, WHI-P131. Superimposition of the interface residues suggested that substitution of Asp 99 (Jak3) into Glu 101 (Jak1) generated steric hindrance and a Tyr 91 to Phe 93 switch altered the shape of catalytic cleft which collectively prohibited the inhibitor binding. Furthermore, in-silico mutagenesis of these two residues back to Asp and Tyr enabled Jak1 to accommodate WHI-P131. These results may provide clues for the design and optimization of selective kinase inhibitors.

The Janus kinases (JAKs) are a family of receptor-associated tyrosine kinases and play important roles in numerous cytokine and growth factor signaling pathways [1]. JAKs constitutively bind to cytoplasmic region of cytokine receptors in the absence of stimulation. Following ligand interaction with its cognate receptor, signal transducers, and activator of transcription (STATs) were phosphorylated by members of Jak family. Activated STATs then dimerize and translocate into the nucleus and promote specific transcription programs [2–4]. In mammals, the JAK family has four members, Jak1, Jak2, Jak3, and Tyk2 each with over 1000 amino acids. These kinases are composed of an N-terminal receptor binding domain (also called FERM domain), a SH2-like domain, a pseudokinase domain important for regulating kinase activity and a carboxyl kinase domain. The functions of JAKs have been extensively studied. Jak1 is involved in IL-2R family, IL-4R family, gp130 receptor family and class II receptor family; Jak2 null mice exhibited embryonic lethality due to failure of erythropoiesis but it is also involved in IL-3, IFN-γ, and GM-CSF signaling [5]. The expression of Jak3 is confined in hematopoietic cells. It binds to common receptor gamma chain γC which associates with IL-2R, IL-4R, IL-7 etc. and regulate lymphoid development [6]. Jak3 has been shown to be highly expressed in most cases of acute lymphoblastic leukemia (ALL) and Jak3 specific inhibitors have been designed for treatment of leukemia [7], immediate hypersensitivity reactions [8] and transplant rejection [9].

Protein phosphorylation is the most common type of post-transcriptional regulation and influences a wide range of biological activities. The design of potent inhibitors for specific kinases has been proved to be an effective solution for various kinds of diseases [10]. Although a plethora of gene and protein sequences have been obtained owing to advances in molecular biology, we still lack accurate structures of proteins for drug design and detailed functional analyses.

Jak1 is a pleiotropic kinase involved in type I and II IFN signaling, IL-7 signaling etc. The absence of Jak1 leads to impaired T cell and B cell production and profound defect in response to IFNs [5]. An accurate 3D model could facilitate understanding in its substrate specificity and kinase–receptor interaction. Here, a comparative modeling method is used to provide a high-quality Jak1 structure of kinase domain (KD) based on the X-ray diffraction structures of Jak2 (2B7A) and Jak3 (1YVJ). The binding mode of ATP with Jak1 KD was investigated by protein–ligand docking simulations. Comparison between Jak1 and Jak3 catalytic core revealed key residues responsible for specificity of WHI-P131, a Jak3 selective inhibitor. These results provide important information on the characteristics of Jak1 KD and offer a starting point for further drug development and biochemical analysis.

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Materials and methods

Homology modeling, molecular simulations and docking studies were performed on a SGI Tezro workstation using InsightII 2000 software package. The reference sequence of Jak1 (NP_002218) was obtained from the National center for biotechnology information (NCBI). The consistent valence forcefield (CVFF) and the Discover 3 simulation module were used for energy minimization and flexible docking. We searched Jak1 homologous entries in the protein data bank (www.rcsb.org) by PSI-BLAST (www.ncbi.nlm.nih.gov/blast/). The PSI-BLAST alignment was further refined by using *structural alignment* in the *Homology* module. This alignment was used for comparative modeling implemented in the *Modeler* module which generates structures by applying spatial restraints and molecular dynamics refinement [11]. After the initial coordinate was optimized by loop refinement, molecular mechanics minimization (steepest descent and conjugate gradient minimizations until the model reached 0.01 kcal/mol Å convergence) was also employed to remove unreasonable regions. The quality of the model was assessed by *Procheck*, *profile-3D* and *Prostat*.

Substrate docking was performed using the *Affinity* module in the InsightII package. The structures of ATP and WHI-P131 were generated and automatically optimized in the *builder* module. Substrate or inhibitors were placed into the catalytic groove; residues within 5 Å of the small molecule were defined as flexible. We first generated 50 structures with Monte Carlo search, the non-bonded method is Quartic VDW (van der waals) with coulombic interactions off and VDW scaled down to 0.1. Simulated annealing was subsequently used to further refine the docking results using cell multipole method, VDW and coulombic scales were 0.1 and finally brought to 1, a brief minimization step was used in the end of the run. All other settings were set to default. Ten complexes were generated in each docking run according to the energy ranking. These poses were evaluated and selected based on the interaction energy, hydrogen bonds, RMSD of the ligand and existing knowledge about the kinase fold. The interaction energy between each residue and inhibitor was measured by using the *evaluate-intermolecular* command in the *Affinity* module with 100 Å cutoff.

Results

A homology model for Jak1 kinase domain

To find homologous proteins in the protein data bank, PSI-BLAST [12], a profile–sequence comparison method was used. Jak2 (2B7A) and Jak3 (1YVJ) were found to be 52.5% and 56.1% identical to Jak1 KD, respectively. The localization of the gaps was further optimized (Fig. 1) after structural alignment. We used *Modeler* to generate homology structures and optimized them by a brief energy minimization in order to remove energy strains in the system.

The proposed Jak1 KD structures were evaluated by the *Prostat* and *Profile-3D* module in the InsightII suite and the Procheck program [13]. The final model was selected based on scores in these evaluations. Prostat checks bond lengths and bond angles for deviation from experimental data. Ramachandran plots were generated by Procheck which examines the stereochemical property of the model. As shown in Table 1, our Jak1 KD model satisfied the requirement of these criteria with 91.4% of the Φ–ψ angles in the favored area and none in the disallowed regions. Only one bond length was over 3-fold deviation from average length. Profile-3D employs a scoring method to evaluate the validity of the fold according to compatibility of sequence into the environment of the residue. Almost all the residues in the scoring plot are above zero which suggested a reasonable folding (Fig. 3). The profile-3D score is close to the expectation score as shown in Table 1 and comparable to those of Jak2 and Jak3.

The structure of Jak1 KD retained the key features of protein kinases [14,15] with a N-terminal lobe composed mainly of β-sheets and a C-terminal lobe of α-helices. These two lobes form a catalytic cleft which catalyzes ATP into ADP and transfer the γ-phosphate to tyrosine residues of substrate proteins. The backbone RMSDs between Jak1–Jak2 and Jak1–Jak3 are 1.127 Å and 0.967 Å, respectively, which show close resemblance to these two homologs.

Computational docking of ATP into Jak1 catalytic pocket

To investigate the detailed interaction mode of ATP, the natural substrate, with Jak1, we used a flexible docking approach which allows the interface residues and the ligand to move in the conformation search. The interface residues include: Leu16-Gly19, Val24, Ala41, and Glu91-Leu94 in β-sheets of the N-lobe, Val73, and Ser98 in turns, Arg142-Asp156 in β-sheet of the C-lobe. After several runs of Monte Carlo search and simulated annealing, we obtained an interaction conformation as shown in Fig. 2. The interaction energy is −138.44 kcal/mol which is composed of −40.81 kcal/mol Vdw energy and −97.63 kcal/mol electrostatic energy. The adenosine moiety is masked by the hydrophobic cleft and the phosphate extends toward the solvent and forms the majority of the hydrogen bonds. As shown in Table 2 and Fig. 2B, the three phosphates forms five hydrogen bonds with Glu18 and Arg142; the amino group in purine base forms a hydrogen bond with side-chain carboxyl group of Glu92 and the hydroxyl group in the ribose forms a hydrogen bond with side-chain carboxyl group of Asp156.

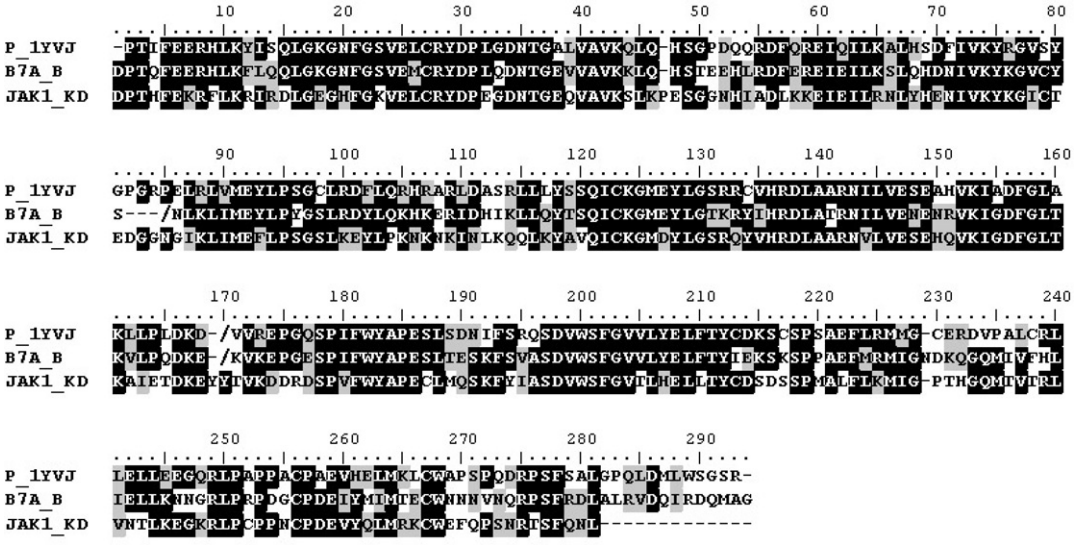

Fig. 1. Sequence alignment of Jak1 KD with Jak2 (B7A_B) and Jak3 (P_1YVJ).